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Prophylactic or therapeutic administration of *Agaricus blazei* Murill is effective in treatment of murine visceral leishmaniasis

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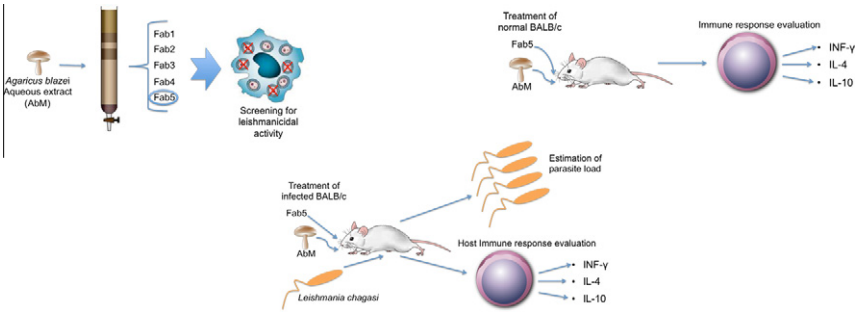
HIGHLIGHTS

- Leishmanicidal activity from fractions purified from *Agaricus blazei* extract (AbM).
- Treatment of BALB/c mice infected with *Leishmania chagasi*.
- Evaluation using parasitological and immunological parameters.
- Efficacy in the prevention and treatment using Fab5 or AbM.
- Therapeutic alternative to visceral leishmaniasis.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study aimed to investigate the *in vitro* antileishmanial activity of five fractions obtained from *Agaricus blazei* water extract (AbM), namely, Fab1, Fab2, Fab3, Fab4, and Fab5; and use the selected leishmanicidal fraction to treat BALB/c mice infected with *Leishmania chagasi*. A curve dose–titration was performed to obtain the concentration to be test in infected animals. In this context, Fab5 fraction and AbM were used in the doses of 20 and 100 mg/kg/day, respectively, with the product been administered once a day. The effect induced by a chemo-prophylactic regimen, based on the administration Fab5 fraction and AbM 5 days before infection, and maintained for an additional 20 days post-infection was compared to a therapeutic regimen, in which the compounds were administered from 0 to 20 days of infection. Control animals were either treated with amphotericin B deoxycholate (AmpB) or received distilled water. All groups were followed up for 10 weeks post-infection, when parasitological and immunological parameters were analyzed. The Fab5 presented the best results of *in vitro* leishmanicidal activity. In the *in vivo* experiments, the use of Fab5 or AbM, as compared to control groups, resulted in significant reduced parasite burdens in the liver, spleen, and draining lymph nodes of the infected animals, as compared to control groups.

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A Type 1 immune response was observed in the Fab5 or AbM treated animals. No significant toxicity was observed. The chemo-prophylactic regimen proved to be more effective to induce theses responses. In this context, the data presented in this study showed the potential of the purified Fab5 fraction of AbM as a therapeutic alternative to treat visceral leishmaniasis. In addition, it can be postulated that this fraction can be also employed in a chemo-prophylactic regimen associated or not with other therapeutic products.

1. Introduction

Leishmaniasis is a group of vector-transmitted diseases that are endemic in 88 tropical and subtropical countries. Many geographic regions are endemic for multiple *Leishmania* species. This is the case in South America, where the disease is caused by at least eight different species of *Leishmania* (World Health Organization, 2009). American tegumentary leishmaniasis (ATL) includes some forms that commonly refer to their clinical and pathologic features: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis (DCL) (Grimaldi and Tesh, 1993). By contrast, human and canine visceral leishmaniasis (VL) in South America is mostly related to infections with *Leishmania chagasi* (Maia and Campino, 2008).

The current treatment for leishmaniasis has been based on the use of pentavalent antimonials. The parenteral administration of these compounds is still the first choice for therapy; however, increased parasite resistance and several side effects are important problems reported by patients (Croft and Coombs, 2003; Minodier and Parola, 2007). Liposomal amphotericin B (AmpB) is effective, but highly cost for the majority of patients (Mondal et al., 2010). Results from clinical trials of oral miltefosine treatment are encouraging; however, therapy is linked to potential toxicity, resistance, and teratogenicity, and should not be given to pregnant or to childbearing age women (Oliveira et al., 2011a). Therefore, the development of new therapeutic strategies to treat leishmaniasis has become a high-priority (Frézard and Demicheli, 2010). In this context, major emphases have been given to the identification of new and lower toxic compounds and alternative administration routes (Garnier and Croft, 2002; Berman, 2005; Giudice and Campbell, 2006; Aguiar et al., 2010; Carneiro et al., 2010).

Agaricus blazei Murill is a commonly found mushroom in Brazil, and its use has been associated with folk medicine in the treatment of some diseases, like leukemia, cancer, and arterial hypertension (Kim et al., 2005; Talcott et al., 2007; Kim et al., 2009). Compounds, such as β-*D*-glucans, glycoproteins, saponins, tannins, cerebrosides, polysaccharides, steroids, such as ergosterol, and fatty acids have been detected in this mushroom, which have been shown to activate and/or modulate the host immune response (Sorimachi et al., 2001; Bernardshaw et al., 2005; Forland et al., 2010). Recently, an *in vitro* antileishmanial activity against *L. amazonensis*, *L. chagasi*, and *L. major* was demonstrated for an *A. blazei* water extract (Valadares et al., 2011).

The present study investigated the antileishmanial activity of five purified fractions of the *A. blazei* water extract, namely, Fab1, Fab2, Fab3, Fab4, and Fab5. For those fractions presenting the best anti-*L. chagasi* activity *in vitro* (Fab4 and Fab5), as well as for the *A. blazei* water extract (AbM), the leishmanicidal activity on intramacrophage *Leishmania*, as well as cytotoxic effect on murine macrophages and human red blood cells, were studied. Afterwards, the Fab5 and AbM were tested *in vivo* using the experimental infection of BALB/c mice with as the model, through comparison of chemoprophylactic and therapeutic regimens. Their effects were also compared with the parenteral administration of AmpB by examining parasitological and immunological parameters. Besides, a phytochemical screening was carried out in the AbM and Fab5, in order to indicate the substances involved in the therapeutic efficacy of the both products.

2. Material and methods

2.1. Mice and parasites

The Committee on the Ethical Handling of Research Animals (CEUA) from the Federal University of Minas Gerais (UFMG) approved all animal handling methods and procedures (code 056/2010). Female BALB/c mice (8 weeks of age) were obtained from the breeding facilities of the Department of Biochemistry and Immunology, Institute of Biological Sciences, UFMG, and were maintained under specific pathogen-free conditions. *L. chagasi* (MHOM/BR/1970/BH46) was grown at 24 °C in Schneider's medium (Sigma, St. Louis, MO, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS, Sigma), 20 mM L-glutamine, 200 U/mL penicillin, and 100 μg/mL streptomycin, at pH 7.4. The soluble *L. chagasi* antigenic extract (SLA) was prepared from a 1×10^{10} stationary-phase promastigotes, as described (Coelho et al., 2003).

2.2. Preparation of the A. blazei water extract and purified fractions

For AbM water extract preparation, 28 g of the fresh mushroom was macerated in 50 mL of milli-Q water containing a protease inhibitor cocktail (Sigma, code P8340). The product was macerated in an ice bath and maintained for 1 h at 4 °C, at which time it was filtered on paper filter to remove the insoluble particles. Lately, the AbM was submitted to centrifugations at 8.000*g* for 45 min at 4 °C, using different Amicon columns with different molecular weight cut-off (daltons – Da). Thus, Fab5 (molecules > 100,000 Da), Fab4 (between 100,000 and 50,000 Da), Fab3 (between 50,000 and 10,000 Da), Fab2 (between 10,000 and 3000 Da), and Fab1 (<3000 Da) were selected. The AbM and the purified fractions were lyophilized and maintained at −80 °C, until use.

2.3. Chemicals

Acetonitrile and methanol (HPLC grade) were purchased from Tedia (Fairfield, OH, USA) and J.T. Baker (Phillipsburg, NJ, USA), respectively. Concentrated phosphoric acid (85% w/v, Merck, Darmstadt, Germany) was used. Ultrapure water was obtained using a Milli-Q plus system (Millipore, Milford, MA, USA). HPLC grade reference substances used were gallic acid (GA, 98%); epigallocatechin (EGC, 90%), catechin (C, min. 98%), epigallocatechin (GC, 98%), epicatechin (EC, 90%); epigallocatechin gallate (EGCG, 95%), purchased from Sigma (Milwaukee, WI, USA).

2.4. Phytochemical analysis and HPLC characterization of AbM and Fab5

The presence of tannins, flavonoids, coumarins, quinones, alkaloids, triterpenes and steroids and saponins was evaluated in AbM and Fab5 by thin layer chromatography (TLC) analysis and specific